Metals as electron acceptors in single-chamber microbial fuel cells

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HIGHLIGHTS

- Fe(III) and Cr(VI) can be used as electron acceptors with high power generation in SCMFCs.
- SCMFC achieved high conversion efficiency of Fe(III) and Cr(VI) as electron acceptors on cathode.
- Anode biofilm communities were similar, but cathode biofilms changed with metals.
- SCMFCs were more effective in metal reduction than anaerobic treatment alone.

ARTICLE INFO

ABSTRACT

Two typical oxidized-status metals (Fe(III) and Cr(VI)) were studied as electron acceptors on cathodes in single chamber microbial fuel cells (SCMFCs) to explore novel sustainable technology for metal treatment. The batch-mode tests indicated that the voltages of SCMFCs steadily increased with Fe(III) concentrations (10, 30, and 50 mg L^{-1}) and Cr(VI) concentrations (1, 3, and 10 mg L^{-1}). The maximum power density was 658 ± 6 mW m^{-2} at 50 mg L^{-1} of Fe(III), and 419 ± 4 mW m^{-2} at 10 mg L^{-1} Cr(VI). The conversion efficiency of Cr(VI) and Fe(III) were high (>89%), and coulombic efficiency ranged 23–100%. Cr(VI) concentration of 10 mg L^{-1} started to irreversibly inhibit SCMFCs. The open circuit potentials (OCPs) well reflected the organic substrate removal in anode and metal reduction on cathode. Cathode liner sweep voltammetry (LSV) showed the electrochemical activity increased with metal concentrations, and the cathode of Fe(III) had better LSV performance than Cr(VI). Microbial community analysis of biofilms showed that the DNA band patterns of anode biofilms were similar, while cathode biofilms varied with electron acceptors. This study demonstrated the high power generation of SCMFCs with metals as electron acceptors, and revealed the great potential of expanding MFCs for diverse waste treatment.

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1. Introduction

Wastewater containing diverse organic substances (e.g. fatty acid, carbohydrate, and protein) and heavy metals (e.g. chromium, copper, and cadmium) has caused severe environmental pollution. Microbial fuel cell (MFC) is a promising biotechnology capable of converting organic substrates in wastewaters (e.g. domestic wastewater, swine wastewater, leachate, and urine) to electricity [1–8]. The electrogenic microorganisms colonized on the anode surface degrade organic substrates and generate electrons, which then transfer to the cathode through external circuit and complete reduction reactions [9]. Two MFC configurations, two-chamber MFCs (2C-MFCs) and single-chamber MFCs (SC-MFCs) have been extensively studied [10–15]. Anode and cathode chambers are separated by membranes (e.g. proton exchange membrane) in 2CMFCs [10], while membranes are removed in SCMFCs and both anode and cathode were contacted with electrolyte (wastewater), with oxygen as cathodic electron acceptor [11,16,17]. Due to the removal of membranes between anode and cathode, SCMFCs have lower internal resistance and higher power generation than 2CMFCs [16]. But oxygen could penetrate through cathode and diffuse into anode solution of SCMFCs, which leads to lower coulombic efficiency than 2CMFCs [9,16].

Oxygen from air has been widely used as the electron acceptor on cathodes in SCMFCs, due to its abundance and high redox potential ($E_{mfc} = 0.805$ V, $pO_2 = 0.2$, pH = 7) [9]. However, to fully

explore SCMFCs as an electro-bio-chemical system to treat wastes, it is important to utilize anode/cathode reactions for oxidizing/reducing diverse contaminants in wastewater. Until now, many studies have focused on the removal of organic substances in anaerobic anodes of 2CMFCs and SCMFCs [1–8,15]. For electron acceptance reactions on cathodes, denitrification (nitrate reduction), fumarate and chlorinated compound reduction were examined in 2CMFCs [18–21]. In fact, metals (e.g. Cr^{6+}, Mn^{7+}, and Fe^{3+}) could also be used as electron acceptors on cathodes, and thus being reduced to less toxic or removed from wastewater [22–24]. But until now, metal reduction has only been studied in 2CMFCs to prevent the transfer of metals from cathode to anode, and to eliminate the potential inhibition on anodic electrogenic bacteria. Besides, the internal resistance (R_{in}) of 2CMFCs is higher than that of SCMFCs, and the cost is high due to membranes and complicated two-chamber structure. Therefore, in order to enhance power generation and simplify MFC configuration for scale up application, it is critical to explore metal reduction in SCMFCs.

This study targeted the reduction of two typical metals, chromium (Cr) and iron (Fe) in SCMFCs. Hexavalent chromium is normally present in wastewaters from electroplating, pigment, and lumber industries [25], and has posed a serious risk to human, animals, and environment due to its high solubility, high toxicity, and potential carcinogenicity [26]. Traditional physical, chemical, and biological processes have been used to reduce Cr(VI) to Cr(III) [27,28], but the problems of excessive chemical usage, high energy consumption, and toxic waste sludge treatment have lowered treatment efficiency and increased the operational costs. In terms of Fe(III), it is abundantly present in the biosphere and some anaerobic bacteria (e.g. *Geobacter* species, *Shewanella* species, *Desulfovibrio vulgaris*, *Thermotoga maritima*) can utilize it as the terminal electron acceptor for their growth [29–31,44], which made it an ideal electron acceptor in MFCs. Effective current-producing anaerobic microorganisms (e.g. *Geobacteraceae, Shewanella*) on the anode are usually Fe(III) reducers [32]. Moreover, both Cr and Fe have high standard potentials ($E_0 = 1.33$ V for Cr from Cr(VI) to Cr(III), $E_0 = 0.77$ V for Fe from Fe(III) to Fe(II), vs SHE), which could increase power generation in MFCs.

In addition, one of the most basic requirements for microbial ecological studies is to identify the microbial diversity. Because the performance of MFCs is closely correlated with the diverse microorganisms (e.g. electrogenic bacteria, anaerobic bacteria for chemical oxygen demand (COD) consumption, and metal reducing bacteria), it becomes necessary to elucidate the community structure diversity in MFCs. Previous studies showed that with higher concentration of organic matters, the microbiota led to higher power generation [3], and the electrogenic potential was dependent on microbial communities through biofilm diversity analysis [55]. In such case, correlating power output with community diversity in MFCs become essential. The species richness and diversity can be primarily screened with methods like RAPD (random amplified polymorphic DNA), which is much simpler than other molecular biological methods [56].

There were four tasks in this study. First, the relationship between power generation and concentration of Cr(VI) and Fe(III) in the SCMFCs was explored, and the maximum Cr(VI) concentration that bacteria could tolerate was determined. Second, the conversion efficiencies and coulombic efficiencies of Cr(VI)/Cr(III) and Fe(III)/Fe(II) in SCMFCs were calculated for the efficiency of using metals rather than oxygen as electron acceptors. Third, important biochemical and electrochemical parameters (e.g. pH, open circuit potential (OCP), and R_{in}) were measured in anode and cathode of SCMFCs to determine the metal impacts. Linear sweep voltammetry (LSV) was performed on cathodes to characterize the electrochemical activity of Cr(VI) and Fe(III). Finally, the biofilms of anode

and cathode were analyzed in this study using RAPD to determine primarily the microbial diversity with different electron acceptors of SCMFCs.

2. Materials and methods

2.1. MFC and electrode materials

Membraneless SCMFCs made of plexiglass bottles (length: 9.5 cm, diameter: 4.5 cm, and total volume of 150 ml) were used in this study. Carbon brush (length: 4 cm, diameter: 4 cm, Mill-Rose Carbon Fiber Brush Anode) fixed on a titanium wire was used as the anode electrode, and plugged into the SCMFC anode chamber. Carbon cloth (effective area: 3 cm^2, 30%wt polytetrafluoroethylene (PTFE), ETek) was used as the cathode and inserted into the glass extension on the lower part of SCMFCs (Fig. 1). The water-side of cathodes was loaded with 0.5 mg cm^{-2} Pt as the cathodic catalyst, and the air-side was coated with three layers of PTFE [16]. Pt was adopted to accelerate the inoculation stage during which electrogenic bacteria would grow on the anode preferentially and steadily. Without Pt catalyst, SCMFCs took twice as long as time for the bacteria growth on electrodes, which would affect the operational time of SCMFCs. After inoculation, the air-side of cathodes was covered with a gasket to prevent oxygen leaking to the water-side (Fig. 1), so that oxygen could not act as the electron acceptor on cathodes, and only metals were electron acceptors.

2.2. Inoculation and SCMFC operation

The SCMFCs were inoculated with municipal wastewater taken from the influent of the Wastewater Treatment Facility at the University of Connecticut, which contained sufficient microorganisms for MFCs. Sodium acetate (20 mmol L^{-1}) was added during batch mode inoculation to provide sufficient carbon substrates for bacterial growth. After 7 days, the voltages of SCMFCs reached 0.25 V at the external resistance ($R_{ext.}$) of 500 Ω, and stabilized during two-month inoculation. Wastewater was then replaced
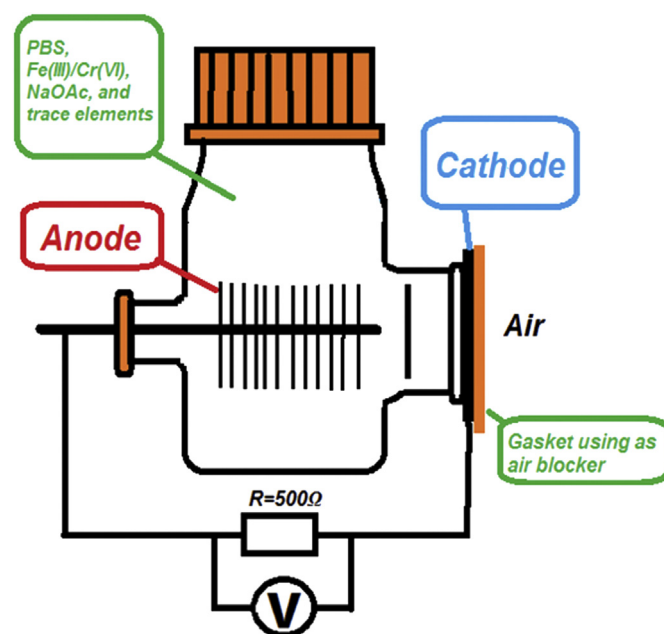

Fig. 1. Configuration of single-chamber microbial fuel cells (SCMFCs) with metal as the electron acceptors.

with phosphate buffer solution (PBS, 50 mmol L^{-1}, pH 6.5) in anode chamber, which contained NH_4Cl 0.31 g, KCl 0.13 g, $NaH_2PO_4 \cdot 2H_2O$ 5.618 g, $Na_2HPO_4.12H_2O$ 6.155 g, 1 ml trace mineral solution and 1 ml trace vitamin per liter solution [33]. Sodium acetate (20 mmol L^{-1}) was added as carbon source for microorganisms. $K_2Cr_2O_7$ and $FeCl_3 \cdot 6H_2O$ solution were also injected to SCMFCs, respectively, to provide Cr(VI) and Fe(III) as electron acceptors. SCMFCs were sparged with N_2 for 10 min to remove the residual dissolved oxygen to prevent the oxygen inhibition to electrogenic bacteria on anodes and the competition with Cr(VI) and Fe(III) as electron acceptors on cathodes. All the experiments were carried out at 30 °C in duplicate at each cycle, and at least two cycles for each metal concentration.

Three concentrations of Cr(VI) (1 mg L^{-1}, 3 mg L^{-1}, and 10 mg L^{-1}) and Fe(III) (10 mg L^{-1}, 30 mg L^{-1}, and 50 mg L^{-1}) were examined individually in batch-mode SCMFCs. Previous studies showed that 1 mg L^{-1} of Cr(VI) was the threshold inhibition concentration for microorganisms in wastewater [34]. Cr(VI) concentrations higher than 1 mg L^{-1} were tested in this study to simulate wastewaters with higher amounts of metals, and to find out whether MFCs could treat higher Cr(VI) concentrations. Fe(III) is not toxic to microorganisms, and the concentrations selected in this study represented the Fe(III) content in diverse conditions (e.g. wastewater, aquatic sediments, and aquifers) [35]. Since the concentrations of Cr(VI) (1 mg L^{-1}, 3 mg L^{-1}, and 10 mg L^{-1}) were lower than Fe(III) (10 mg L^{-1}, 30 mg L^{-1}, and 50 mg L^{-1}), a cycle of Cr(VI) was five days until the voltage dropped below 0.050 V (R_{ext}, 500 Ω), while a cycle of Fe(III) was seven days. Thus, the power production of SCMFCs was studied at 2 days (48 h) in a cycle when the reactions actively proceeded. In addition, because both Cr(VI) and Fe(III) were completely reduced on the 5th day (120 h) after being added in SCMFCs, the metal conversion efficiency (%) was measured after 120 h. Moreover, to elucidate whether other mechanisms like adsorption or sedimentation also contributed to metal removal or reduction in SCMFCs, a control test was conducted on SCMFCs added with Cr(VI) and Fe(III) without being connected with external circuit throughout the test period.

2.3. Analysis and calculations

2.3.1. Power generation
The voltage across R_{ext} (500 Ω) was continuously recorded every 0.5 h using a Keithley 2700 data logging system. Polarization curve measurement was conducted with different R_{ext} (10–2940 Ω) and the voltage over each R_{ext} was measured with a multimeter (Radioshack digital multimeter) until the reading stabilized after 5 min. The current density was calculated according to $I = V/(R_{ext} \times A)$, and the power density was calculated according to $P = V^2/(R_{ext} \times A)$, where A is the effective area of the cathode.

2.3.2. The anodic and cathodic open circuit potentials(OCPs), pH, internal resistance(R_{in}) and coulombic efficiency
The open circuit potentials (OCPs) of anode and cathode were measured using a potentiostat (Gamry P600) with Ag/AgCl (+197 mV vs SHE) as the reference, anode or cathode as the working electrodes, and Pt wire as the counter electrode. pH was measured using a portable pH meter (Thermo Fisher Scientific Orion 3-star). The R_{in} was calculated as the slope of V–I curves generated from the polarization measurement excluding the higher voltages, which were the activation loss region [17].

The coulombic efficiency was calculated as the ratio of the current flowing across SCMFCs and the theoretical current based on the total Cr(VI) and Fe(III) fed into the system.

$$CE = \frac{M \int_0^t I dt}{nFV(C_o - C_t)} \times 100\%$$

where M is the molecular weight (52 for Cr and 56 for Fe); I is the current(A); n is the number of electrons accepted by metals on cathodes (3 for Cr (Cr^{6+} to Cr^{3+}), and 1 for Fe (Fe^{3+} to Fe^{2+})); F is Faraday's constant (9.64853×10^4 C mol^{-1}); V is the liquid volume of SCMFCs (L); and C_0 is the initial concentration(g L^{-1}) of metals fed into system and C_t is the concentration(g L^{-1}) after time (t).

Microscopy observation of biofilm growth and metal deposition on the electrodes was carried out with Scanning Electron Microscope (SEM) (Joel6335F). The chemical elements on the electrode surfaces were analyzed using the Energy-dispersive X-ray spectroscopy (EDX). Prior to SEM observation, the electrode samples were taken from SCMFCs and treated as previously described [36].

2.3.3. Linear sweep voltammetry (LSV)
The electrochemical analysis of cathodes of SCMFCs was performed using linear sweep voltammetry (LSV) in a three-electrode configuration, with Ag/AgCl as the reference, cathodes as the working electrodes, and Pt wire as the counter electrode. The potentiostat (Gamry P600) was used to perform the LSV tests at a scan rate of 0.25 mV s^{-1} and scanned from the cathode OCP to −0.45 V.

2.3.4. Cr(VI) and Fe(III) measurement
Colorimetric standard methods with a spectrophotometer (Varian Cary 50 Bio UV–visible spectrophotometer) were used to measure Cr(VI) and Fe(II) concentrations. Specifically, 1,5-diphenycarbazide method was used for the concentration of soluble Cr(VI) by filtering the samples through 0.2 μm membrane syringe filters (Fisherbrand nylon) [37]. Fe(II) concentration was determined using 1,10-phenanthrolin colorimetric method [37], and Fe(III) was then calculated from the difference between total Fe concentration and Fe(II) concentration.

2.3.5. Microbial community analysis of biofilms on anodes and cathodes
RAPD analysis was conducted for the biofilms growing on anodes and cathodes of the SCMFCs with Cr(VI) and Fe(III) as the electron acceptors and the control SCMFC without adding any metal solution (oxygen as electron acceptor). Upon the completion of SCMFC operational tests (90 days), biofilms were collected from anodes and cathodes. The DNA of these biofilms was extracted with the soil DNA isolation kit (MO BIO Laboratories, Carlsbad). The extracted DNA was amplified using 4 different random primers. The sequences of the primers were: 57-5′GGCCAACGCGGCC3′, 58-5′CCTGCAGCAGA3′, 59-5′CMCGYCRSCA 3′, 60-5′CAGCAGCAGCAG 3′ (Amersham) for the environmental samples to characterize the variability without specificity. Amplification was performed in a total volume of 25 μl solution, containing template 2.5 μl, primer 2.5 μl, Master Mix 12.5 μl (AmpliTaq Gold 360 Master Mix, Applied Biosystems), 360 GC enhancer 1.25 μl (AmpliTaq Gold 360 Master Mix, Applied Biosystems), and water 1.25 μl (Fisher Science). RAPD was carried out under the following conditions: single cycle of first denaturation at 94 °C for 10 min, 45 circles containing 1 min s denaturation at 95 °C, 1 min annealing at 45 °C, and 1 min extension at 72 °C, and one circle of final extension at 72 °C for 10 min [57]. Amplification products were analyzed by electrophoresis in 1.5% agarose gels and detected by staining with SYBR safe (Invitrogen). The total band numbers of four primers were counted to determine the diversity of the microbial community on anodes/cathodes under different electron acceptors (Fe, Cr, and oxygen).

Base on the diversity index of RAPD, the gel image was analyzed using the PyElph software in which the presence and absence of bands were designated as 1 and 0 by comparing with the DNA ladder. The analysis showed the clusters in terms of different primers used.

3. Results and discussion

3.1. Power generation of SCMFCs fed with Cr(VI) and Fe(III)

For the SCMFCs fed with Fe(III), power density steadily increased with Fe(III) concentrations (Fig. 2a) and the maximum power generation of SCMFCs was 658 ± 6 mW m^{-2} at the concentration of 50 mg L^{-1}. Higher concentration of Fe(III) led to higher power density, due to lower R_{in}, higher ionic strength, and higher electron transfer capacity [17,38]. Besides, Fe is an excellent terminal electron acceptor for anaerobic chemotrophic microorganisms [30]. SCMFCs reached the highest current density at the R_{ext} of 10 Ω (the lowest R_{ext} used in this study) (Fig. 2a), since low R_{ext} increased the current production. Like power density, the current density increased with Fe(III) concentrations, and reach 0.46 mA cm^{-2} at 50 mg L^{-1}.

For the SCMFC fed with Cr(VI), power density increased with Cr(VI) concentration and the maximum power generation of

Fig. 2. Voltage and power generated in the SCMFCs with different concentrations of Fe(III)(a) and Cr(VI)(b) when external resistance on the system is changed from 10 to 2940 Ω at 48 h. ((a)V 10 represented voltage at the concentration of 10 mg L^{-1}, V 30 represented voltage at the concentration of 30 mg L^{-1}, V 50 represented at the concentration of 50 mg L^{-1}, PD 10 represented power density at the concentration of 10 mg L^{-1}, PD 30 represented at the concentration of 30 mg L^{-1}, PD 50 represented at the concentration of 50 mg L^{-1}; (b) V 1 represented voltage at the concentration of 1 mg L^{-1}, V 3 represented voltage at the concentration of 3 mg L^{-1}, V 10 represented at the concentration of 10 mg L^{-1}, PD 1 represented power density at the concentration of 1 mg L^{-1}, PD 3 represented at the concentration of 3 mg L^{-1}, PD 10 represented at the concentration of 10 mg L^{-1}.).

SCMFCs was 419 ± 4 mW m^{-2} at the concentration of 10 mg L^{-1}(Fig. 2b). However, due to the toxicity of Cr(VI), the high power generation of Cr concentration 10 mg L^{-1} was only achieved in the 1st cycle (Fig. 2b), while stable power generation still continued in the 2nd cycle at Cr(VI) concentrations of 1 mg L^{-1} and 3 mg L^{-1}. The highest current density was achieved at the R_{ext} of 10Ω, with 0.27 mA cm^{-2} for 10 mg L^{-1}, 0.24 mA cm^{-2} for 3 mg L^{-1}, and 0.23 mA cm^{-2} for 1 mg L^{-1}. These results demonstrated that before reaching to toxic level, increasing metal concentrations enhanced the power generation of SCMFCs. Metal concentrations were closely correlated with bacterial metabolic activities [30,34], especially for anaerobic chemotrophic microorganisms with slow growth rate and using oxidized metal ions as electron acceptor to obtain energy [30]. Cr(VI) of 1 mg L^{-1} was found toxic for microorganisms [34], but this study showed that SCMFCs had stable power generation at Cr(VI) 1–3 mg L^{-1}, and even 10 mg L^{-1}, indicating that electrogenic bacteria could still produce power even at high toxic metal concentrations. In addition, the maximum power generation of SCMFCs in this study was higher than 2CMFCs fed with $Cr_2O_7^{2-}$ (55.5 mW m^{-2} and 123.4 mA/m^2 at 80 mg L^{-1} [39], and 150 mW m^{-2} and 0.04 mA cm^{-2} at 200 mg L^{-1} [23]). This clearly demonstrated that even though 2CMFCs tolerated higher metal concentrations than SCMFCs, SCMFCs had much higher power generation than 2CMFCs.

3.2. Reduction of metals in SCMFCs

The conversion efficiency (%) of Cr(VI) and Fe(III) were examined after 5-day (120 h) treatment (Table 1), during which the voltages of SCMFCs were sustainably high. SCMFCs had high conversion efficiency ($>89.0 \pm 1.0\%$) for both metals, which indicated that using electrons generated from anaerobic anodic reactions to reduce Cr(VI) and Fe(III) on cathodes was quite efficient. In addition, the oxygen redox potential (ORP) of −420 to −450 mV (data not shown) in SCMFCs demonstrated that there was no oxygen present in anode chamber, and the reduction of metals was responsible for electron acceptance on cathode. It should be noted that the conversion efficiency of Cr(VI) increased with concentrations, which was related with the measurement methods that spectrophotometers can only measure Cr(VI) concentration as low as 0.1 mg L^{-1}. Thereby, the conversion efficiency at low concentration (Cr(VI) 1 mg L^{-1}) was underestimated. The maximum reduction percentage of Cr(VI) (98.7% at 10 mg L^{-1}) was higher than the phy-chemical processes. However, phy-chemical processes had a shorter time (92% for electrocoagulation at 20 mg L^{-1} during 50 min [40], 91% for biosorption at 30 mg L^{-1} during 120 min [41], 97% for electrocoagulation and electroflotation at 10 mg L^{-1} during 75 min [42], and 96% for nanofiltration at 17 mg L^{-1} during 900 min [43]) than SCMFCs. This indicated that using SCMFCs to reduce metals is an effective bioprocess but requiring longer retention time than traditional phy-chemical processes.
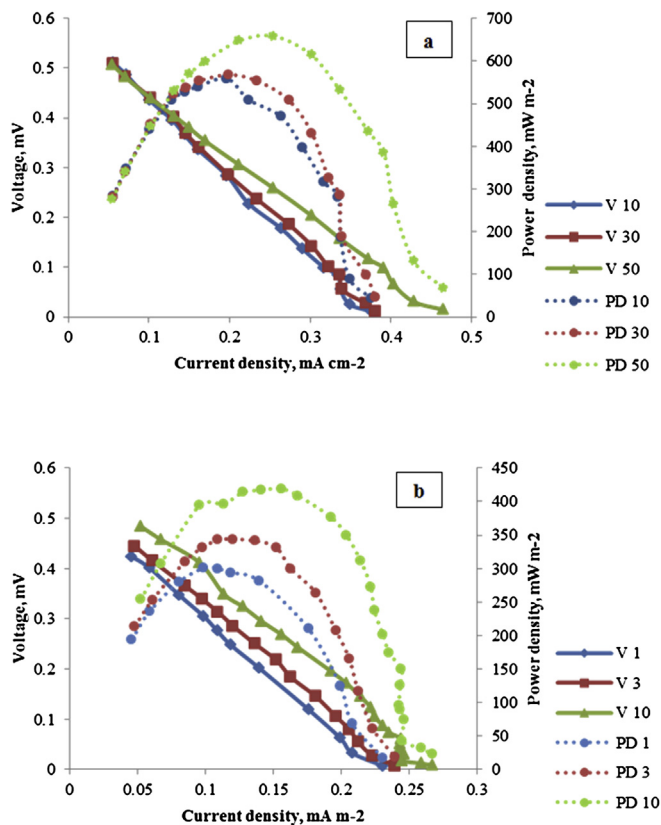
Table 1
Conversion efficiency (%) of Cr(VI) and Fe(III) in SCMFCs during 120 h treatment.

Initial Cr(VI) concentration (mg L^{-1})	Final Cr(VI) concentration (mg L^{-1})	Conversion efficiency (%)
1.10 ± 0.10	≤ 0.1	89.0 ± 1.0
3.00 ± 0.10	≤ 0.1	95.7 ± 0.4
10.0 ± 0.20	≤ 0.1	98.8 ± 0.1

Initial Fe(III) concentration (mg L^{-1})	Final Fe(III) concentration (mg L^{-1})	Conversion efficiency (%)
10.0	0.28 ± 0.08	97.2 ± 0.8
30.0	2.10 ± 0.56	93.0 ± 1.9
50.0	2.72 ± 0.92	94.6 ± 1.8

Mechanisms other than electron reduction on cathodes could also contribute to the concentration change of Cr(VI) and Fe(III) in SCMFCs. In the control tests of SCMFCs without being connected with external circuit, about 88% of conversion efficiency was observed at the Cr(VI) of 1 mg L^{-1} (Table 2). There were two possible reasons for metal reduction or removal in the control tests. First, a wide range of aerobic and anaerobic bacteria (e.g. *Pseudomonas dechromaticans*, *Bacilli, Clostridia*) could reduce Cr(VI) to Cr(III). There were also possibilities that electrogenic bacteria (e.g. *Geobacter* and *Shewanella*) could reduce Cr(VI) since most of them are Fe(III) reducers [31]. Second, some species (e.g. *Pseudomonas aeruginosa*) could adsorb heavy metals like Cr(VI) [39]. The EDX results clearly demonstrated the deposition of metals on cathodes (Figs. 3 and 4). However, the conversion efficiency of the control tests (SCMFCs without being connected with the external circuit) decreased at higher concentration of Cr(VI) (63% at 3 mg L^{-1} and 28% at 10 mg L^{-1}) (Table 2), which was in the opposite trend of the operated SCMFCs (93% at 3 mg L^{-1} and 98% at 10 mg L^{-1}) (Table 1). This clearly showed that SCMFCs effectively reduce Cr(VI) and harvest energy simultaneously, while metal reduction was inhibited in the control tests operated at anaerobic environment alone. In terms of Fe, when the Fe(III) concentration was lower than 30 mg L^{-1}, the conversion efficiencies in the control tests (98% at 10 mg L^{-1} and 92% at 30 mg L^{-1}) (Table 2) were similar to those of the operated SCMFCs (97% at 10 mg L^{-1}, and 93% at 30 mg L^{-1}) (Table 1), mostly due to anaerobic bacterial metabolisms and meal adsorption, since most active electrogenic microorganisms in MFCs were Fe-reducing bacteria [30,32]. However, at high Fe(III) concentration (50 mg L^{-1}), the conversion efficiency was only 79% in the control tests, much lower than that of the operated SCMFCs (94%), indicating that the rate of anaerobic digestion was much slower than that of electrochemical reactions. This is a clear evidence that SCMFCs have higher metal conversion efficiency than traditional anaerobic processes, and can utilize metal reduction to harvest electricity.

3.3. Changes of cathode surface properties during metal reduction in SCMFCs

Biofilm growth on cathodes normally occurred in SCMFCs treating wastewater [6,45,46]. It had been found that biofilms on cathodes facilitated electron acceptance and enhanced power generation [6,39,46], while the high extents of cathodic biofilm growth would lower the cathode OCPs [6,36]. Compared with the clean Pt-loaded weaving carbon cloth (Fig. 3a), the cathodes in SCMFCs treating metals clearly had biofilms and metal crystals (Fig. 3b and c), which indicated the attached bacteria could carry out the metal reduction on cathodes. The elements on the cathodes analyzed using EDX showed the Pt peak on the clean Pt-loaded cathodes (Fig. 3a), while Cr and Fe peaks appeared on the
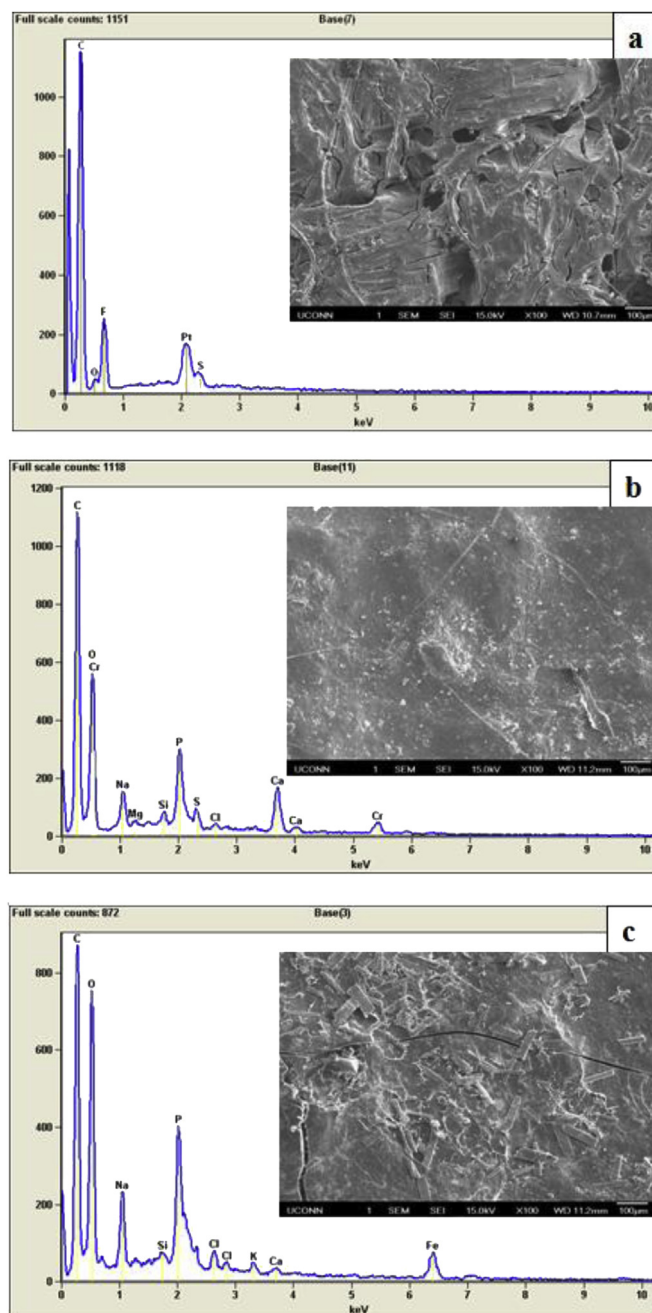

Fig. 3. The SEM pictures and EDX spectrums of cathodes in SCMFCs before and after operation (a. clean Pt loaded cathode; b. cathode operated with Cr(VI) solution; c. cathode operated with Fe(III) solution).

Table 2
Conversion efficiency (%) of Cr(VI) and Fe(III) in the control SCMFCs without being connected with external circuit during 120 h.

Initial Cr(VI) concentration (mg L^{-1})	Final Cr(VI) concentration (mg L^{-1})	Conversion efficiency (%)
1.1 ± 0.10	≤0.1	88.0 ± 1.0
3.0 ± 0.10	1.10 ± 0.20	63.3 ± 6.7
10.0 ± 0.20	7.20 ± 0.85	28.4 ± 6.9

Initial Fe(III) concentration (mg L^{-1})	Final Fe(III) concentration (mg L^{-1})	Conversion efficiency (%)
10.0	0.15 ± 0.05	98.5 ± 0.50
30.0	2.60 ± 0.70	92.0 ± 2.33
50.0	10.4 ± 1.36	79.2 ± 2.72

cathodes treating metals (Fig. 3b and c), indicating the metal deposition on cathodes. Previous studies found that metal deposition (e.g. Cr, Fe, Ca, Cu) led to cathode fouling, blocked the contact between catalysts (e.g. Pt used in this study) and electrolyte solution in MFCs, increased R_{in}, and decreased power generation [47,48]. Therefore, cathode surfaces needed to be cleaned periodically to remove metal deposition.

Compared with the clean carbon brush anode (Fig. 4a), bacteria attachment and substrate deposition on the carbon brush were observed (Fig. 4b and c). The EDX results showed that carbon peaks were predominant but there were still other element peaks when treating with metals (Fig. 4), indicating that Cr, Fe and other
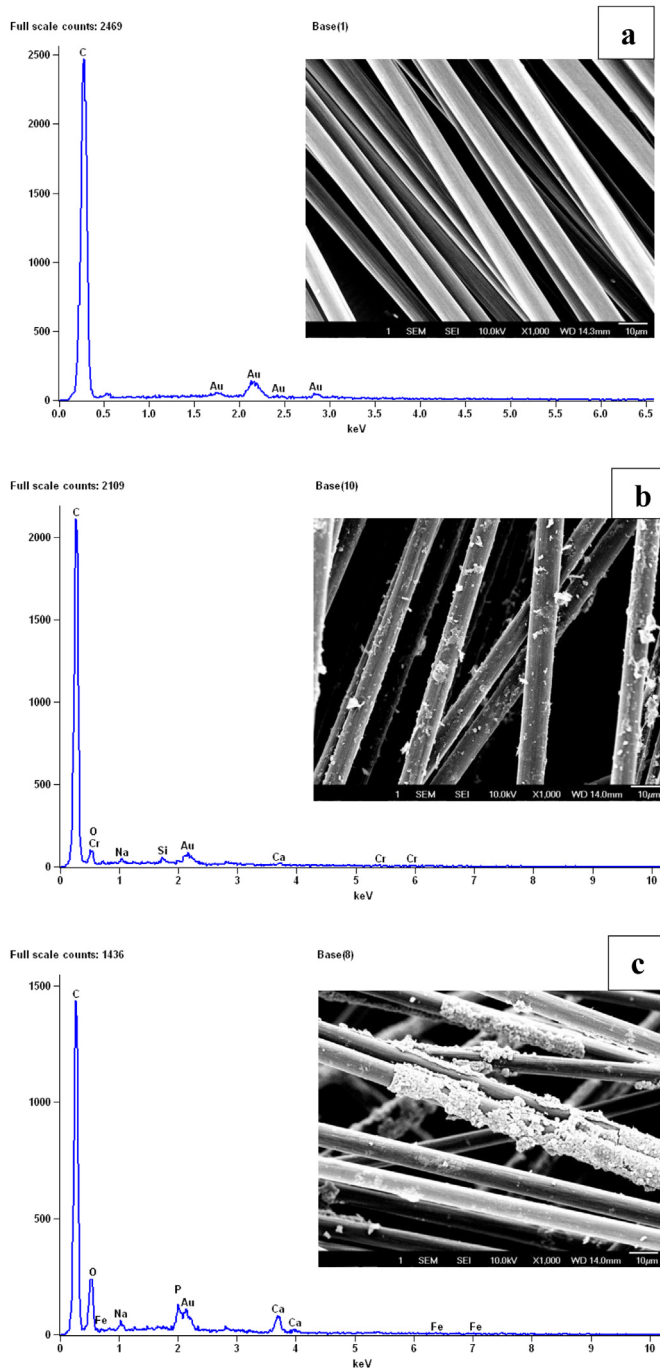
Fig. 4. The SEM pictures and EDX spectrums of anodes in SCMFCs before and after operation (a. clean carbon brush anode; b. anode operated with Cr(VI) solution; c. anode operated with Fe(III) solution).
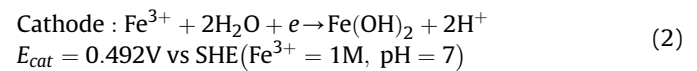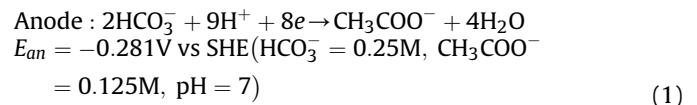
components (e.g Na, P, Ca) in the solution were adsorbed on the carbon brush. The Cr (5-6 KeV) and Fe peaks (6—8 KeV) were not obvious compared with those from cathode EDX (Fig. 3b and c), indicating that most of these metals were deposited on the cathodes rather than anodes. The element Au in the EDX was from the gold sputtering for samples to make it more conductive.

3.4. Electrochemical characteristics of metal reduction in SCMFCs
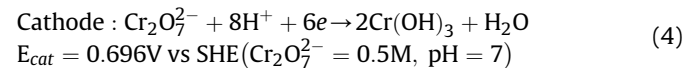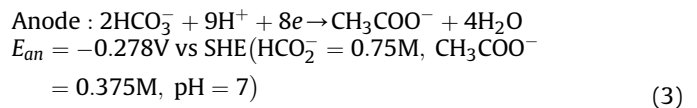
The reduction of Cr(VI) and Fe(III) on cathode was determined using the anode/cathode half—cell reactions (Eq. 1—4). The predominant species in the half—cell reactions were determined generally by E-pH diagram (not shown). The calculation of the theoretical maximum electromotive force (EMF) (Eq. (5)) was conducted based on the stoichiometric relationships between Cr(VI) and acetate/Fe(III) and acetate, in which the molar concentration of acetate oxidized (the organic substrate in anode) should be 0.125 times of Fe(III) and 0.375 times for Cr(VI) (Eq. 1—4). Therefore, under specific conditions (pH 7, 1 bar and 298.15 K), the theoretical electrode reduction potential of 1 M of Fe(III) was 0.492 V (vs. SHE) and a complete oxidation of 0.125 M of acetate has a reduction potential of −0.281 V(vs. SHE). The combination of these two half reactions generate a total electromotive force of 0.773 V (vs. SHE) (Eq. (5)). Following the same calculations, the reduction potential of 1 M Cr(VI) is 0.696 V (vs. SHE), the reduction potential of complete oxidation of 0.375 M sodium acetate is −0.278 V (vs. SHE), and hence the total EMF for Cr(VI) is 0.974 V (vs. SHE).

For SCMFCs containing Fe(III):

$$\text{Anode}: 2HCO_3^- + 9H^+ + 8e \rightarrow CH_3COO^- + 4H_2O$$
$$E_{an} = -0.281V \text{ vs SHE}(HCO_3^- = 0.25M, \ CH_3COO^- = 0.125M, \ pH = 7) \tag{1}$$

$$\text{Cathode}: Fe^{3+} + 2H_2O + e \rightarrow Fe(OH)_2 + 2H^+$$
$$E_{cat} = 0.492V \text{ vs SHE}(Fe^{3+} = 1M, \ pH = 7) \tag{2}$$

For SCMFCs containing Cr(VI):

$$\text{Anode}: 2HCO_3^- + 9H^+ + 8e \rightarrow CH_3COO^- + 4H_2O$$
$$E_{an} = -0.278V \text{ vs SHE}(HCO_2^- = 0.75M, \ CH_3COO^- = 0.375M, \ pH = 7) \tag{3}$$

$$\text{Cathode}: Cr_2O_7^{2-} + 8H^+ + 6e \rightarrow 2Cr(OH)_3 + H_2O$$
$$E_{cat} = 0.696V \text{ vs SHE}(Cr_2O_7^{2-} = 0.5M, \ pH = 7) \tag{4}$$

The cell electromotive force (E_{emf}) was calculated as

$$E_{emf} = E_{cat} - E_{an} \tag{5}$$

The electrochemical measurement showed that the overall OCP (~630 mV) of the SCMFCs with Fe(III) was about 0.8 times as the theoretical values (773 mV) (Table 3) and the overall OCP (~580 mV) of the SCMFCs with Cr(VI) was about 0.6 time as the theoretical values (974 mV) (Table 3), indicating that electrochemical reactions of electron transfer from the external circuit and metal reduction proceeded effectively on cathodes. It should be noted that the anodic OCPs of all SCMFCs tested were −487 ± 4 mV vs Ag/AgCl (−290 ± 4 mV vs SHE) (Table 3), which was around the theoretical value of sodium acetate oxidation (~−284 mV vs SHE). This elucidated that the electrogenic bacteria growing on the anode effectively generate electrons from anaerobic degradation of sodium acetate and were not affected by the metals added in SCMFCs.

The internal resistance (R_{in}) of SCMFCs fed with Cr(VI) (708—749 Ω) was higher than those with Fe(III) (393—507 Ω), and dropped with metal concentrations (Table 3), since higher metal concentrations decreased the ohmic losses by increasing the solution conductivity and ionic strength [10]. Due to Cr(VI) concentrations (1—10 mg L^{-1}) much lower than Fe(III) concentrations (10—50 mg L^{-1}), the corresponding R_{in} of Cr(VI) were high. The R_{in} of SCMFCs in this study was higher than those (30—300Ω) with air cathode [17,49]. There might be three reasons. First, the cathode

Table 3
Cathode OCPs, pH, R_{in}, and coulombic efficiency for SCMFCs during 120hr treatment with different initial concentration of Fe(III) and Cr(VI).

Initial concentration (mg L^{-1})	Fe(III)10	Fe(III)30	Fe(III)50	Cr(VI) 1	Cr(VI) 3	Cr(VI)10
Anode potential (mV)	-484 ± 1	-485 ± 2	-488 ± 2	-486 ± 3	-489 ± 1	-485 ± 2
Cathode potential (mV)	135 ± 2	141 ± 3	163 ± 3	96.0 ± 6	106 ± 5	116 ± 4
Open circuit potential (OCP) (mV)	619 ± 1	626 ± 5	651 ± 5	582 ± 3	594 ± 4	601 ± 2
Initial pH	6.49	6.46 ± 0.01	6.47 ± 0.01	6.47 ± 0.02	6.48 ± 0.01	6.45 ± 0.01
Final pH	6.34 ± 0.01	6.42 ± 0.02	6.43 ± 0.01	6.3 ± 0.01	6.37 ± 0.02	6.35 ± 0.01
Internal resistance (Ω)	507	486	393	749	726	708
Coulombic efficiency (%)	>100	37.5	23.4	>100	83.6	25.6

area (carbon cloth, 3 cm^2) was much smaller than anode (carbon brush), which limited the reduction reactions on cathodes. Previous studies found that cathode rather than anode is the limiting factor for power generation of SCMFCs [49]. Second, the precipitation of Cr(III) or Fe(II) (e.g. Cr(OH)$_3$, Fe(OH)$_2$, Fe(OH)$_3$) and thick biofilms on cathodes (Fig. 3b and c) would impede the continuous electron transfer and increase R_{in}. Third, the concentration of PBS solution (50 mmol L^{-1}) used in this study was lower than other SCMFC tests (200 mmol L^{-1}) [49].

The coulombic efficiency decreased substantially with the increase of metal concentrations (Table 3). At low concentrations (Fe 10 mg L^{-1}, Cr 1 mg L^{-1}), the coulombic efficiency was higher than 100%, which indicated that there should be other electron sinks present in SCMFCs, such as the residual trace oxygen in solution and other minerals (e.g. Cu^{2+}, MnO$_7^{4-}$) in mineral solution. These extra electron sinks led to the overestimation of electron acceptance efficiency (coulombic efficiency value) at low metal concentrations. However, with the increase of the concentration of Cr(VI) and Fe(III), these multiple sinks could be ignored since the residual oxygen would be consumed quickly and the amount of trace elements added were much lower than Cr(VI) and Fe(III) contents. In addition, other oxidation states of Cr (e.g. Cr(II), Cr(IV), and Cr(V)) could be the intermediate product existing in the SCMFC solution but they are unstable intermediates. Cr(III) was the most stable oxidation state [54], which acted as the main final electron acceptors in cathodic reactions. As for Fe, the standard reduction potential of Fe(III) to Fe was -0.04 V (vs SHE), which could not happen simultaneously in SCMFCs. Therefore, the calculation of coulombic efficiency could be simplified by using Cr(VI) to Cr(III) or Fe(III) to Fe(II) as the electron acceptor and disregarding other contributors.

At high concentrations (Fe 50 mg L^{-1} and Cr 10 mg L^{-1}), the coulombic efficiency values were 23.4% and 25.6%, respectively (Table 3). In contrast, the conversion efficiencies for Fe(III) and Cr(VI) remained high (>93%) at high concentrations (Table 1). This discrepancy revealed that some processes (e.g. anaerobic digestion, bacterial assimilation uptake, and adsorption) rather than electron acceptance reduction contributed to Cr(VI)/Fe(III) concentration change, but not electricity generation. It should be noted that the coulombic efficiency values calculated in this study was based on metal reduction on cathodes, unlike those based on organic substrate degradation on anodes [3,11,16]. The coulombic efficiency values obtained (23−100%) was much higher than those with air cathodes (13−27%) [3,11,16], which clearly verified that the easily dissolved metals (e.g. Cr and Fe) have much lower mass transfer resistances for cathodic reactions than oxygen diffusion from air, and metal reductions had lower overpotentials than oxygen reduction reaction [48].

pH of SCMFCs dropped (6.3−6.4) after 120-hr operation (Table 3), due to the proton generation from sodium acetate degradation in anode (Eqs. (1) and (3)). The pH drop also indicated that sodium acetate (20 mmol L^{-1}) was sufficient for electrons needed by metal reduction on cathodes throughout batch-mode operation. Even though the reduction of Cr(VI) on the cathode

consumed more protons than provided from anodic reactions (Eq. (4)), the precipitation of Cr^{3+} at pH 6-7 also produced protons (Eq. (6)) [39] and avoided the proton shortage problem.

$$2Cr^{3+} + 7H_2O \rightarrow 2Cr(OH)_3 + 6H^+ + H_2O \qquad (6)$$

3.5. Metal reduction activities on cathodes measured by LSV

The effects of metal concentrations to the cathode electrochemical performance were evaluated using LSV measurement. The current produced in LSV is the flow of electrons needed to support the active electrochemical processes at the rate consistent with the potential [50]. The cathode at higher metal concentration had steeper wave slope of the I–V curves, and the highest electrochemical reaction rates achieved at the highest concentrations of Cr(VI) (10 mg L^{-1}) and Fe(III) (50 mg L^{-1}) (Fig. 5). This indicated that higher concentration of metals accelerated the reduction reaction on cathodes, which corresponded well with the lower R_{in} at higher metal concentration (Table 3). In addition, because Cr(VI) existed as Cr$_2$O$_7^{2-}$ and Fe(III) existed as Fe^{3+} or Fe(OH)$_3$, the mole concentration of Cr(VI) was almost half of Fe(III) at 10 mg L^{-1}, so that its electrochemical reaction rate was lower than Fe(III) (10 mg L^{-1}, Fig. 5). Moreover, the relationship of electron flux (J) and current (i) could explain the LSV phenomenon (Eq. (7)) [50]. The current (i) is proportional to the flux of electroactive species (e.g. Cr(VI) and Fe(III) tested) transported to the electrode surface ($x = 0$), $J_O(0,t)$. The flux is also proportional to the concentration gradient (C_O (x,t)) of oxidized species at the electrode surface. Here, n is the number of electron exchanged in the reaction, F is Faraday's constant (9.64853×10^4 C/mol), A is the electrode surface area, and D_O is the diffusion coefficient. Eq. (7) clearly showed that higher concentration possesses higher electrochemical reaction rate. Thereby, the SCMFCs fed with Fe(III) exhibited much higher
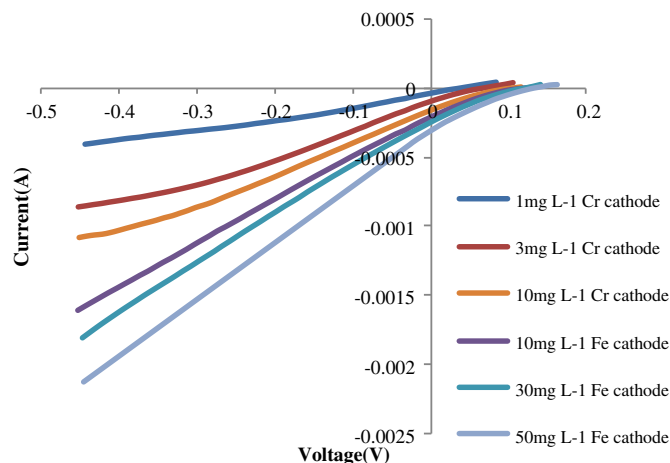

Fig. 5. Cathode liner sweep voltammetry (LSV) curves of different concentration of Fe(III) and Cr(VI) after 120 h.

electrochemical reaction rates than those with Cr(VI), which was the result of the higher concentration of Fe(III) than Cr(VI).

$$-J_O(0,t) = \frac{i}{nFA} = D_O \left[\frac{\partial C_O(x,t)}{\partial x}\right]_{x=0} \tag{7}$$

3.6. Microbial communities of biofilms on anodes and cathodes

The DNA band patterns of anode biofilms with 3 different electron acceptors were similar (Fig. 6a). The sum of the band numbers with 4 primers was the same for Cr and oxygen, a bit fewer for Fe (Fig. 6a and Table 4). The dendrogram constructed with a neighbor joining method from PyElph also demonstrated that the electron acceptors with the same primer was related but two primers (No.57 and No.58) showed Fe and oxygen had a closer distance than Cr (Fig. 7a). No.59 primer showed a closer distance of Cr and oxygen and No.60 showed a closer distance of Cr and Fe. This indicated that all the SCMFCs were inoculated with the same wastewater and the bacterial species were similar for the anodes. The electrochemically active bacteria (e.g. *Geobacter* and *Shewanella* species) and others species symbiotically related to these bacteria were integral and similar on anode biofilms regardless of the electron acceptors were adopted [59]. In addition, although Cr(VI) was toxic for the bacterial growth, microbial community structures were not changed (Figs. 6 and 7a), indicating that the Cr(VI) concentrations (≤ 10 mg L^{-1}) was durable for electrogenic bacteria on the anodes and the SCMFCs could still produce power with the presence of Cr. This implied that the electrochemically

Table 4
RAPD bands of biofilms at anodes and cathodes in SCMFCs.

Primer	Anode band number			Cathode band number		
	Cr	Fe	O$_2$	Cr	Fe	O$_2$
57	2	1	1	6	5	4
58	7	4	5	4	6	6
59	4	3	5	5	4	5
60	1	1	3	1	5	3
Total	14	9	14	16	20	18

active bacteria on the anode which were usually considered to be iron-reducing bacteria (e.g. *Geobacter* and *Shewanella* species) could tolerate other metals (e.g. Cr, Mn, U) [31]. For Fe, the band number was a little fewer than those with Cr(VI) and oxygen, indicating that the chosen concentration (10–50 mg L^{-1}) was high enough to affect some species growth on the anode (Table 4).

The RAPD results of cathode microorganisms showed that the cathodes with Fe(III) had the most bands (20) (Table 4) among the cathodes tested. The dendrogram with the neighbor joining
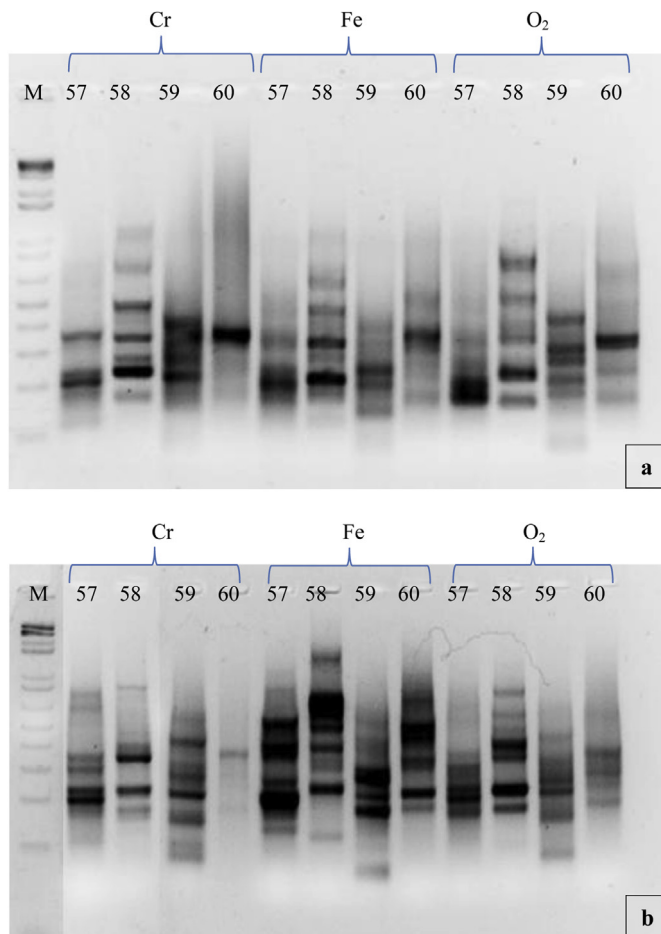
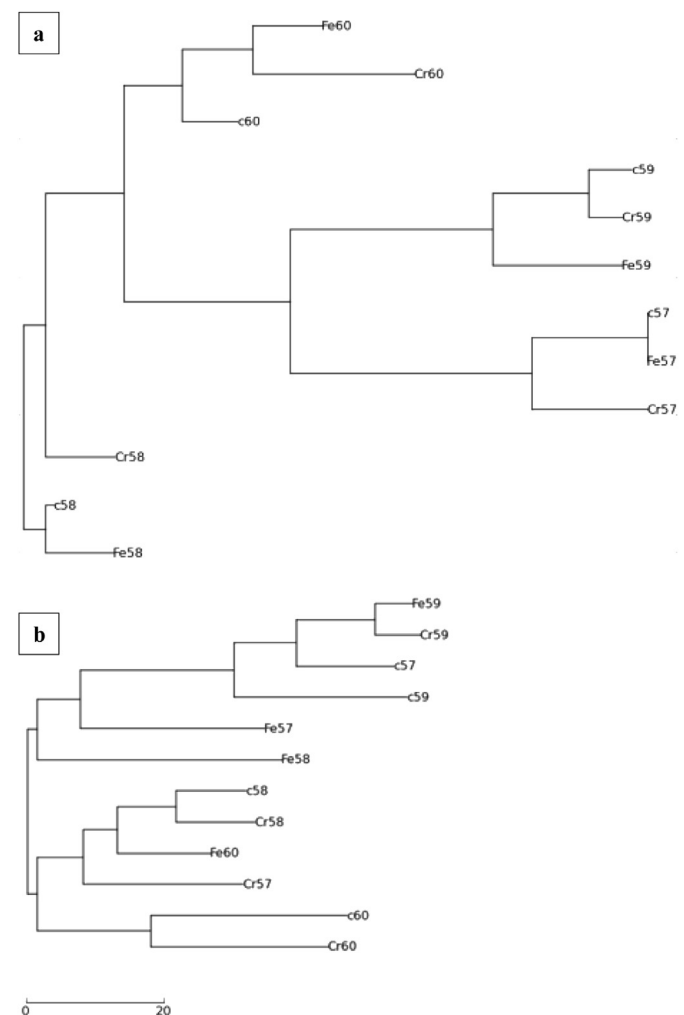**Fig. 6.** RAPD results of biofilms on anodes (a) and cathodes (b) in SCMFCs.

Fig. 7. The dendrogram constructed with neighbor joining method (a. anode biofilm dendrogram with different electron acceptors in terms of 4 primers: Cr57/58/59/60 refers to Cr(VI) as electron acceptor in the MFCs, Fe57/58/59/60 60 refers to Fe(III) as electron acceptor, c57/58/59/60 60 refers to oxygen as electron acceptor; b. cathode biofilm dendrogram with different electron acceptors in terms of 4 primers: Cr57/58/59/60 refers to Cr(VI) as electron acceptor, Fe57/58/59/60 60 refers to Fe(III) as electron acceptor, c57/58/59/60 60 refers to oxygen as electron acceptor.).

method showed that the band patterns with different electron acceptors were not consistent with the same primer (Fig. 7b). Previous studies had found that some bacteria capable of reducing Cr(VI) were used as the biocatalyst for Cr(VI) reduction in MFCs [39,46,52,53]. In addition, for MFCs using oxygen as the electron acceptor, bacteria could be used as the biocatalyst for oxygen reduction reaction (ORR) [6,58]. ORR possessed the higher redox potential (0.805 V, at pH 7 and pO_2 0.2) than Fe(III) and Cr(VI), thus oxygen as electron acceptor could produce higher power density (766 mW m^{-2}) [16] than Fe(III) and Cr(VI). However, the RAPD results showed that the diversity for oxygen was not significantly different from those for metals, indicating that the power generation was not much affected by bacterial diversity. The different patterns demonstrated that cathode bacteria varied with different electron acceptors. Overall, the RAPD test results revealed the fundamental idea of the microbial diversity in MFCs. In order to clearly differentiate the bacterial species, the microbial community analysis of 16sRNA gene based clone experiment should be conducted to identify the bacteria on anode and cathode in MFCs.

3.7. Significance of metal reduction in SCMFCs

This study, for the first time, applied the metal reduction in SCMFCs to expand the applications for treating diverse wastes. Stable power generation was achieved in SCMFCs at various concentrations of Cr(VI) and Fe(III), indicating that using oxidized-status metals as electron acceptors on cathode is a cost-effective approach to treat toxic metals and generate electricity. Compared with 2CMFCs, SCMFCs had much simpler structure, easy installation, and high power generation for future scale-up application in wastewater treatment. Moreover, because some metals (e.g. Fe and Mn) are widely distributed in natural water bodies and sediment, metal reduction on cathodes in this study reveals a great potential of applying benthic MFCs in underwater energy harvest [51]. However, several important issues should be solved for large-scale operation of metal reduction in MFCs. First, even though SCMFCs had higher power generation than 2CMFCs, they had lower tolerance of toxic metals than 2CMFCs [23,39]. Possible solution might be genetically transferring DNA from metal-tolerance bacteria to electrogenic bacteria to enhance the tolerance, or developing novel MFC configurations to minimize the impacts of toxic metals on anodic bacteria. Second, power generation of SCMFCs should be further enhanced by lowering R_{in}, increasing cathode area, using higher PBS concentration, and/or removing metal precipitation on the cathode. Finally, the retention time of the batch-mode SCMFCs was as long as 7 days in this study, which was much longer then the contact time (several hrs) of conventional physical–chemical processes [40–43]. Continuous flow MFC tests should be conducted to determine the optimal retention time for anaerobic treatment of metals and provide the guidance for scale-up MFCs for real-world application.

4. Conclusion

Metal reduction on cathodes in SCMFCs was studied at different concentrations of Cr(VI) and Fe(III). Metal conversion efficiency and power generation were correlated. The inhibition of toxic Cr(VI) on anode/cathode was elucidated. Four major conclusions were drawn from this study.

First, metal concentrations posed two-side effects on SCMFCs performance. Higher metal concentration generally led to high power generation, but Cr(VI) at 10 mg L^{-1} started to inhibit power generation of SCMFCs. Fe(III) concentrations did not have negative impacts on SCMFCs.

Second, the conversion rates of Cr(VI)/Cr(III) and Fe(III)/Fe(II) were high (>89%), indicating that metals were good electron acceptors on cathodes.

Third, the LSV tests demonstrated that cathode electrochemical activity increased with metal concentrations, which well corresponded with R_{in} of SCMFCs.

Fourth, microbial community analysis of biofilms using RAPD indicated that the anode biofilms were similar with different metals in SCMFCs, while the cathode biofilms changed with electron acceptors.

Acknowledgments

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